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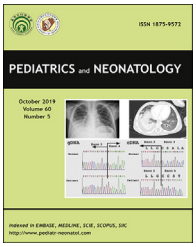

Original Article

Cortisol production in preterm infants with or without late-onset adrenal insufficiency of prematurity: A prospective observational study

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Key words
adrenal cortex;
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Background: Immature adrenocortical function in preterm infants may cause inadequate production of cortisol under stress, resulting in adrenal insufficiency of prematurity (AOP). The objective of this study is to compare cortisol production in preterm infants with and without late-onset AOP.
Methods: Of 27 preterm infants born at less than 32 weeks gestation, cortisol production was analyzed in those who did (patients, group P) and did not (controls, group C) eventually develop late-onset AOP. Blood samples were prospectively collected every two weeks after birth, and steroid hormone concentrations in the pathway to cortisol production were measured retrospectively.
Results: We restricted the initial subjects to infants with gestation less than 29 weeks to adjust for confounding factors, culminating in matched infants in groups P ($n = 8$) and C ($n = 11$). The cortisol concentrations did not differ between the groups before AOP onset ($P = 0.20$), but the total concentrations of precursors for cortisol were higher in group P ($P < 0.0001$). The total concentrations of precursors in group C were inversely correlated with postmenstrual age ($\rho = -0.38$, $P < 0.01$). The pattern of changes in total concentrations of precursors differed between the groups ($P < 0.05$).
Conclusion: Adrenal cortex maturity in preterm infants develops in parallel with postmenstrual age. Infants with late-onset AOP have undeveloped maturation of adrenocortical function after birth.

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1. Introduction

The fetal adrenal cortex changes structurally and functionally with gestational age and maturation of adrenocortical function. In the fetal zone, which is a large part of the fetal adrenal cortex, the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) required to produce cortisol is low, while steroid sulfotransferase activity is high. Thus, cortisol biosynthesis is suppressed and dehydroepiandrosterone sulfate (DHEA-S) is mainly produced.[1–4] After 23 weeks gestation, 3β-HSD is expressed in the definitive and transitional zones of the fetal adrenal cortex, and cortisol production increases after late gestation (32–36 weeks).[3–5] However, in preterm infants, adrenocortical function is not mature and cortisol is not adequately secreted in response to stress, leading to glucocorticoid-responsive circulatory collapse due to adrenal insufficiency of prematurity (AOP).[6–8] While cortisol production is regulated by the hypothalamic-pituitary-adrenocortical (HPA) axis, and cortisol secretion might be lower in some ill preterm infants because of inactivity of the extremely premature brain,[9] more recent data indicate that the primary problem of AOP is immaturity of the adrenal cortex.[10]

Most cases of glucocorticoid-responsive hypotension in preterm infants develop within the first week of life.[10–12] However, in preterm infants, late-onset glucocorticoid-responsive hypotension can be induced by mild stress after the first week of life and sometimes causes severe neurological damage.[13–15] In our previous case-control study, cortisol secretion was not low in preterm infants with late-onset glucocorticoid-responsive hypotension, but concentrations of cortisol precursors increased, suggesting that late-onset circulatory collapse caused by AOP might be a result of relative adrenal insufficiency under stress.[16] However, all samples from infants with late-onset AOP were collected at the time of onset. Thus, it is unclear whether these infants had limited function to produce cortisol before AOP onset, and developmental changes in the adrenal gland before and after AOP onset.

The maturity of adrenocortical function is related to the duration of gestation,[17] but the timing of fetal adrenal involution in preterm infants is uncertain.[18,19] Thus, we hypothesized that maturity of the adrenal cortex in preterm infants is related to postmenstrual age, and that those with late-onset AOP have limited function to produce cortisol until the corresponding age of late gestation, 32–36 weeks postmenstrual age. To verify this hypothesis, we prospectively investigated the course of adrenocortical function maturity based on steroid hormone concentrations in preterm infants with and without late-onset AOP.

2. Methods

2.1. Subjects

The entry criteria were preterm infants born at less than 32 weeks gestational age without life-threatening morbidities at birth, glucocorticoid treatment before onset of AOP, surgery, and major congenital or chromosomal anomalies. Written informed consent from parents was obtained to collect blood samples at hospitalization. The gestational age was determined by obstetric ultrasonography and the menstrual period. Small for date (SFD) was defined as body weight <10th percentile of the normal birth weight curve at each gestational age. The Clinical Risk Index for Babies (CRIB) II score was used to quantify the severity of illness.[20] Antenatal steroid treatment (ANS) was defined as administration of two 12-mg doses of betamethasone to accelerate fetal lung maturity. Infants whose mothers received antenatal steroids were not excluded because the effects of ANS on cortisol production are limited after the first week of life.[21] Two groups were defined: preterm infants who received glucocorticoids for late-onset circulatory collapse due to AOP in the neonatal intensive care unit (NICU) (patients, group P), and those who did not receive glucocorticoids in the NICU (controls, group C).

For each infant, we measured the concentrations of steroid hormones, cortisol, cortisone, DHEA, 17-OH-pregnenolone, pregnenolone, DHEA sulfate (DHEA-S), 17-OH-pregnenolone sulfate (17-OH-pregnenolone-S), pregnenolone sulfate (pregnenolone-S), 17-OH-progesterone, and progesterone. The ratios of cortisol/17-OH-progesterone and cortisol/cortisone were calculated to estimate the activities of 21α-hydroxylase and/or 11β-hydroxylase, and 11β-hydroxysteroid dehydrogenase (11β-HSD), respectively.[17]

2.2. Criteria for adrenal insufficiency

The pathophysiology of adrenal insufficiency in preterm infants is uncertain, and this makes it difficult to establish diagnostic criteria. Furthermore, delayed intervention for late-onset glucocorticoid-responsive circulatory collapse leads to the occurrence of severe neurological impairment, including periventricular leukomalacia. Therefore, we have developed guidelines for appropriate glucocorticoid treatment in our NICU,[16] based on the work of the Japanese Study Group for Neonatal Endocrinology, in which tentative diagnostic criteria were proposed for late-onset glucocorticoid-responsive circulatory collapse.[15] In our criteria, replacement hydrocortisone (1–2 mg/kg) is given when at least two of volume- and pressure-resistant hypotension, oliguria, hyponatremia, lung edema, and increased demand

for oxygen treatment are present in a preterm infant after the first week of life. Adrenal insufficiency is diagnosed when all signs improve within 6 h without other supportive treatment, after ruling out other causes of circulatory collapse, such as infection, patent ductus arteriosus and hypovolemia. We note that respiratory instability is included because a strong association of adrenal insufficiency with development of bronchopulmonary dysplasia has been found in preterm infants.[7] Volume- and pressure-resistant hypotension is defined as systolic blood pressure <40 mmHg and/or mean blood pressure <80% of that in the previous stable state, despite aggressive volume resuscitation and dopamine and/or dobutamine administration beyond the conventional dose (3–10 μg/kg/min); oliguria as urine output of <0.5 mL/kg/h over the past 8 h or <1.0 ml/kg/h over the past 24 h; and hyponatremia as serum sodium <130 mEq/L or a rapid decrease of >5 mEq/L. Lung edema is diagnosed by chest X-ray. Increased oxygen treatment is defined as a rapid increase of >0.1 in the fraction of inspired oxygen.

2.3. Sample collection

Blood samples of 200 μL were obtained every two weeks after birth at routine check-ups until discharge or the expected date of delivery. Samples were drawn between 9 a.m. and 4 p.m. by venipuncture. To avoid placental and parturition effects on steroid production and cortisol surge, samples obtained during the first week of life were excluded from analysis. Samples obtained within 24 h after glucocorticoid treatment in group P were also excluded. All samples were centrifuged immediately, and serum was stored at −70 °C until assay.

2.4. Assay of steroid hormones

Determination of serum concentrations of steroid hormones was performed in a blinded manner.[16,22] Serum samples (100 μL) were treated with 0.6% aqueous glutamic acid and separated into fractions of unconjugated and sulfo-conjugated steroids on a solid-phase extraction column. DHEA, 17-OH-pregnenolone, pregnenolone, 17-OH-progesterone, progesterone, cortisone, and cortisol (unconjugated steroids) were purified by HPLC, and their serum levels were quantified by enzyme immunoassay, except for those of cortisol and cortisone, which were determined by GC/MS. DHEA-S, 17-OH-pregnenolone-S, and pregnenolone-S (sulfo-conjugated steroids) were hydrolyzed with aryl-sulfatase and purified by HPLC. The free steroids were derivatized with *N,O*-bis (trimethylsilyl)-trifluoroacetamide and analyzed by GC/MS. Results are expressed as the monosulfate concentration in serum. The intra- and inter-assay coefficients of variation in these assays were <6.6% and <15.6%, respectively.

2.5. Sample size calculation

In our previous study,[16] the amount of total precursor hormones that were substrates for 3β-HSD in infants with late-onset AOP differed significantly in patients at the time of onset compared to controls, whereas the cortisol concentrations did not differ between the two groups. Detection of this difference between the groups at a power of 80% and a two-sided alpha level of 0.05 required an enrollment of 4 infants in group P and 12 infants in group C, assuming an incidence of late-onset AOP of 25% among the study infants.[14] To compare steroid hormone concentrations between groups P and C, each individual should be clustered because multiple blood samples were collected from the same individual. As the intra-cluster correlation coefficient was not available from our previous study,[16] the value was assumed to be a maximum of 0.02.[23] The number of blood samples in a cluster was assumed to be 5 in the study period, and the effective sample size (the number of subjects actually enrolled in the study) was 4 infants in group P and 13 infants in group C, giving a total of 85 blood samples in the study.

2.6. Statistical analysis

Clinical characteristics were assessed using a Fisher exact test for categorical variables and a Mann–Whitney *U* test for continuous variables. Steroid hormone levels were evaluated by non-parametric methods due to non-normal distributions. Comparisons of steroid hormone levels between groups P and C were made by Mann–Whitney *U* test. Variables with a potential association with occurrence of late-onset AOP were assessed in univariate and multivariate logistic regression analyses. Correlations between postmenstrual age and steroid hormone levels in group C were assessed using a Spearman rank correlation test. Effects of postmenstrual age and late-onset AOP on levels of cortisol and precursors in groups P and C were evaluated using linear mixed models, which take into account serial correlation within participants due to repeated measurements and incorporate randomly missing data. Random intercept models were used to evaluate the interaction between AOP onset and postmenstrual age. Statistical analyses were performed using JMP Pro 13 (SAS Institute Inc., Cary, NC, USA), with a significant difference defined as $P < 0.05$.

2.7. Study approval

The parents of all subjects gave written informed consent to participation in the study. The study was approved by the Ethics Committee of Tokyo Women's Medical University.

3. Results

3.1. Patient characteristics

Of 76 preterm infants born before 32 weeks gestational age who were admitted to the NICU at Tokyo Women's Medical University between October 2006 and March 2008, 56 were initially eligible for the study. The 20 excluded cases were infants with congenital heart disease (n = 4, single ventricle, pulmonary valve stenosis, tetralogy of Fallot, coarctation of aorta), severe intraventricular hemorrhage (n = 4), chromosomal anomaly (n = 2, trisomy 18), severe cardiac shock due to accidental out-of-hospital delivery (n = 3), patent ductus arteriosus ligation surgery (n = 1), glucocorticoid

Table 1 Baseline characteristics in the infants.

	Total n = 27	Group C n = 19	Group P n = 8	*P*
Gestational age (wk)*	28.6 (25.4, 31.4)	28.9 (25.4, 31.4)	26.1 (25.4, 28.7)	<0.05
Birth weight (g)*	984 (412, 1644)	1141 (441, 1644)	810 (412, 1118)	<0.01
SFD	6 (22%)	3 (16%)	3 (38%)	0.32
Gender (boy/girl)	20/7	13/6	7/1	0.63
Apgar score at 5 min*	8 (3, 9)	8 (6, 9)	8 (3, 9)	0.57
CRIB II score*	9 (2, 14)	7 (2, 13)	10 (9, 14)	<0.01
Antenatal steroid	22 (81%)	16 (84%)	6 (75%)	0.62
Histological CAM	10 (37%)	5 (26%)	5 (63%)	0.10
Cesarean section	23 (85%)	17 (89%)	6 (75%)	0.56

Group C, controls; group P, patients; SFD, small for date; CRIB II score, the clinical risk index for babies II score; CAM, chorioamnionitis.
Values are presented as number (percentage) except when marked as follows: * median (range).
P values depict the differences between groups P and C (Fisher exact test for categorical factors, Mann–Whitney *U* test for continuous variables).

Table 2 Selected baseline characteristics and outcomes in the infants <29 weeks gestational age.

	Total n = 19	Group C n = 11	Group P n = 8	*P*
Gestational age (wk)*	26.7 (25.4, 28.9)	27.7 (25.4, 28.9)	26.1 (25.4, 28.7)	0.28
Birth weight (g)*	924 (412, 1290)	1006 (441, 1290)	810 (412, 1118)	0.062
SFD	4 (21%)	1 (9%)	3 (38%)	0.26
Gender (boy/girl)	14/5	7/4	7/1	0.34
Apgar score at 5 min*	8 (3, 9)	8 (6, 9)	8 (3, 9)	0.69
CRIB II score*	9 (7, 14)	8 (7, 13)	10 (9, 14)	0.15
Antenatal steroid	15 (79%)	9 (82%)	6 (75%)	1.0
Histological CAM	9 (47%)	4 (36%)	5 (63%)	0.37
Cesarean section	16 (84%)	10 (91%)	6 (75%)	0.55
Postnatal age at AOP onset (d)*			18 (8, 39)	
Postmenstrual age at AOP onset (wk)*			30.1 (27.0, 31.1)	
RDS treated with surfactant	10 (53%)	5 (45%)	5 (63%)	0.65
BPD at 28 days of life	15 (79%)	7 (64%)	8 (100%)	0.10
BPD at 36 weeks postmenstrual age	8 (42%)	4 (36%)	4 (50%)	0.66
PDA treated with indomethacin	10 (53%)	5 (45%)	5 (63%)	0.65
IVH	1 (5%)	1 (9%)	0	1.0
PVL	3 (16%)	1 (9%)	2 (25%)	0.55
ROP requiring laser	10 (53%)	6 (55%)	4 (50%)	1.0
Sepsis	3 (16%)	0	3 (38%)	0.058

Group C, controls; group P, patients; SFD, small for date; CRIB II score, the clinical risk index for babies II score; CAM, chorioamnionitis; RDS, respiratory distress syndrome; BPD, bronchopulmonary dysplasia; PDA, patent ductus arteriosus; IVH, intraventricular hemorrhage; PVL, periventricular leukomalacia; ROP, retinopathy of prematurity.
Values are presented as number (percentage) except when marked as follows: * median (range).
P values depict the differences between groups P and C (Fisher exact test for categorical factors, Mann–Whitney *U* test for continuous variables).

treatment before onset of AOP (n = 5), and death at birth (n = 1). Parents did not consent to the study in 26 cases, 20 did not receive steroid treatment, and 10 received steroid treatment after the first week of life due to late-onset AOP. However, one control was excluded due to intraventricular hemorrhage and hydrocephalus complications, 1 patient died of sepsis 10 days after birth, and another patient with a gestational age of 27 weeks and 4 days was excluded because continuous blood sampling was difficult due to severe fetal growth restriction (birth weight: 346 g). Of the 26 infants whose parents did not agree to take part in the study, 6 received steroid treatment after the first week of life due to late-onset AOP. Finally, 27 preterm infants were enrolled in the study, with 8 in group P and 19 in group C. The patients recovered from all signs of adrenal insufficiency after a single dose of glucocorticoids.

The baseline characteristics and outcomes of the subjects are summarized in Table 1. Those in group P had a lower gestational age, lighter birth weight, and higher CRIB II score, compared to those in group C. The incidences of common neonatal morbidities, such as respiratory distress syndrome treated with surfactant, bronchopulmonary dysplasia at 28 days of life and 36 weeks postmenstrual age, patent ductus arteriosus treated with indomethacin,

intraventricular hemorrhage, periventricular leukomalacia, retinopathy of prematurity requiring laser therapy, and sepsis did not differ between the groups (data not shown).

The CRIB II score includes the gestational age, birth weight, sex, temperature at admission, and base excess of the infant. Given the multicollinearity among these variables associated with occurrence of late-onset AOP, CRIB II score, ANS, and SFD were assessed in logistic regression analyses as relevant variables.[24] In univariate analyses, CRIB II score (OR 1.67 per score; 95% CI, 1.06–2.63; $P = 0.007$) was significantly associated with late-onset AOP. ANS tended toward a decreased risk and SFD tended toward an increased risk of late-onset AOP, but without significance. In multivariate analysis, only CRIB II score (OR 1.65 per score; 95% CI, 1.03–2.64; $P = 0.014$) was significantly associated with late-onset AOP, as in univariate analysis.

To adjust for confounding factors, we restricted initial samples to infants born before 29 weeks gestation (Table 2). In contrast to the entire population, these selected infants were well matched. Infants in group P (n = 8) did not differ from infants in group C (n = 11) in population characteristics and clinical outcomes. The median postnatal and postmenstrual ages for late-onset AOP were 18 days (range, 8–39 days) and 30.1 weeks (range, 27.0–31.1 weeks), respectively.

3.2. Steroid hormone concentrations before AOP onset

As all occurrences of late-onset AOP were less than 32 weeks postmenstrual age, the concentrations of steroid hormones in group P before AOP onset were compared with those in group C at less than 32 weeks postmenstrual age (Table 3). The 3β-HSD enzyme has reduced activity in preterm infants. Therefore, the levels of precursor hormones that serve as 3β-HSD substrates were compared between the groups.[16,25] DHEA and DHEA-S are not direct substrates for cortisol but were measured because they are the main products from precursors in the fetal adrenal gland. Progesterone and 17-OH-progesterone, precursor hormones downstream of 3β-HSD, were also examined. The cortisol concentrations did not differ significantly between the groups, but those of DHEA-S and 17-OH-pregnenolone-S were significantly higher in group P ($P < 0.0001$, $P < 0.05$, respectively), and pregnenolone-S showed a tendency to be higher in group P ($P = 0.076$). There were no significant differences in the levels of DHEA, 17-OH-pregnenolone, pregnenolone, 17-OH-progesterone, progesterone and cortisone, or in the cortisol/17-OH-progesterone and cortisol/cortisone ratios between the groups.

3.3. Association of late-onset AOP with undeveloped function of the adrenal cortex

Most precursor hormones that are substrates for 3β-HSD in group P were at higher levels than expected. Due to variability in the groups, the amount of precursors was calculated as the concentration of DHEA, 17-OH-pregnenolone, pregnenolone and their sulfates for comparison between the groups. To avoid effects of repeated measurements and confounding factors, the patterns of changes in cortisol and precursor concentrations were compared using linear mixed models. Relationships of late-onset AOP with cortisol and precursor levels before and after late-onset AOP at each week of postmenstrual age were examined (Fig. 1). The pattern of changes in cortisol concentrations did not differ between the groups ($P = 0.055$, Fig. 1A), and those in group C were not correlated with postmenstrual age ($\rho = -0.10$, $P = 0.50$, Fig. 1A). The levels of precursors significantly decreased in group C as postmenstrual age increased ($\rho = -0.38$, $P < 0.01$, Fig. 1B). The pattern of changes in concentrations of cortisol precursors differed significantly between the two groups ($P < 0.05$, Fig. 1B), and the precursors showed value convergence as postmenstrual age increased. The concentrations of precursors in group P were significantly higher than those in group C before AOP onset (median, interquartile range: 3.8,

Table 3 Comparison of steroid hormone concentrations between the pre-AOP onset in group P and the <32 weeks postmenstrual age in group C.

	Group C, <32 wks PMA n = 11	Group P, pre-AOP onset n = 8	*P*
Number of samples	19	13	
Cortisol (nmol/L)	347 (250, 582)	482 (378, 670)	0.20
Cortisone (nmol/L)	162 (103, 243)	173 (128, 226)	0.79
DHEA-S (μmol/L)	2.7 (0.83, 5.2)	7.5 (6.3, 11)	<0.0001
17-OH-pregnenolone-S (μmol/L)	0.29 (0.10, 0.51)	0.57 (0.23, 0.79)	<0.05
Pregnenolone-S (μmol/L)	0.73 (0.28, 0.82)	1.1 (0.51, 1.9)	0.076
DHEA (nmol/L)	4.0 (0.31, 19)	1.6 (0.77, 5.5)	0.49
17-OH-pregnenolone (nmol/L)	10 (1.3, 29)	17 (5.6, 44)	0.59
Pregnenolone (nmol/L)	9.2 (3.3, 16)	6.3 (5.5, 10)	0.50
17-OH-progesterone	5.6 (0.027, 33)	1.9 (0.14, 18)	0.56
Progesterone	0.031 (<0.01, 4.1)	<0.01 (<0.01, 7.1)	0.56
Cortisol/17-OH-progesterone ratio[a]	0.46 (0.15, 182)	0.21 (0.32, 27)	0.40
Cortisol/Cortisone ratio	1.9 (0.088, 2.4)	0.46 (0.039, 1.5)	0.21

DHEA, dehydroepiandrosterone; AOP, adrenal insufficiency of prematurity; PMA, postmenstrual age; -S, sulfate.
Values are presented as median (interquartile range) or number of samples.
P values were calculated by Mann–Whitney *U* test.
[a] Ratio divided by 100 for readability.

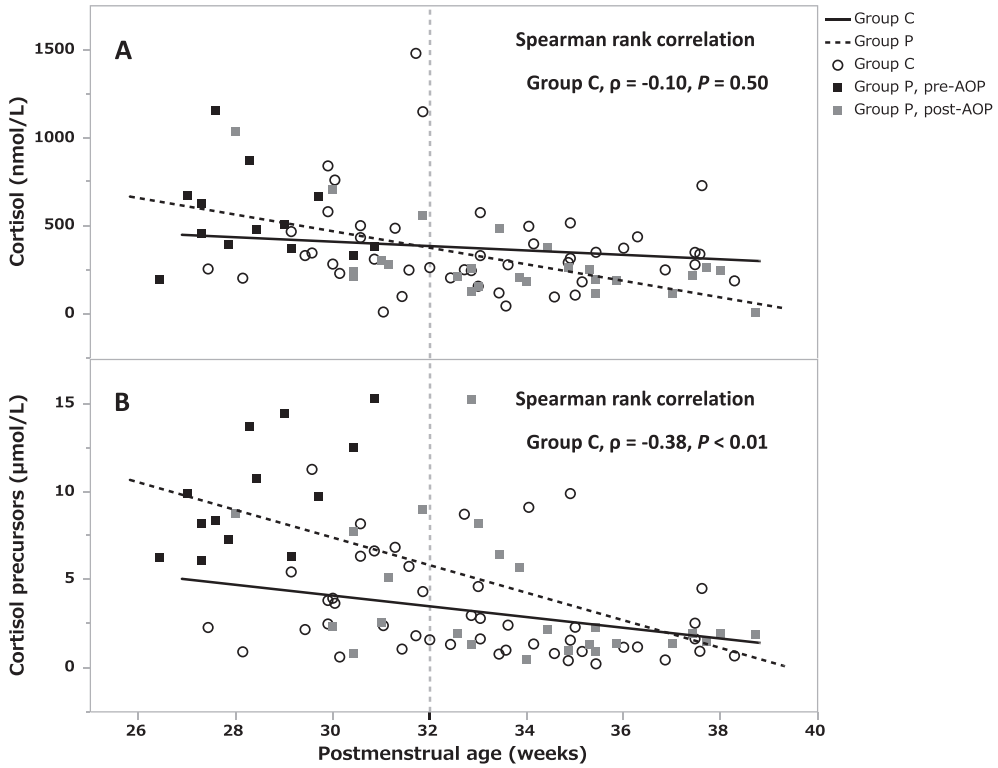

Figure 1 Correlation of postmenstrual age with cortisol and total precursors to cortisol in preterm infants with and without late-onset AOP. Dehydroepiandrosterone, 17-OH-pregnenolone, pregnenolone, and their sulfates are included as cortisol precursors via the enzyme 3β-hydroxysteroid dehydrogenase. Open circles (n = 46) and solid lines show data and linear regression for group C, and closed squares (black, pre-AOP onset, n = 13; gray, post-AOP onset, n = 25) and dotted lines show the results for group P. **A,** Cortisol concentrations in group C were not correlated with postmenstrual age (Spearman rank correlation; $\rho = -0.10$, $P = 0.50$), and the pattern of changes in cortisol concentrations did not differ between the two groups ($P = 0.055$). **B,** Concentrations of cortisol precursors in group C were significantly correlated with postmenstrual age (Spearman rank correlation; $\rho = -0.38$, $P < 0.01$), and the pattern of changes in concentrations of cortisol precursors did differ significantly between the two groups ($P < 0.05$). The dotted vertical line represents the boundary of 32 weeks postmenstrual age.

2.2—6.3 vs. 9.7, 6.8—13 μmol/L, $P < 0.0001$) but were reduced to the level in group C after 32 weeks postmenstrual age.

4. Discussion

In this prospective observational study, cortisol concentrations did not differ between preterm infants with and without AOP, despite the infants with AOP having significantly higher accumulation of cortisol precursors before AOP onset. Moreover, these associations were eliminated by the expected date of delivery, while maturity of the adrenal cortex in preterm infants was related to postmenstrual age. These results indicate immature cortisol production in preterm infants who developed late-onset AOP until 32 weeks postmenstrual age, and they show that immaturity of adrenocortical function resolved as postmenstrual age increased.

Fetal cortisol biosynthesis is suppressed due to reduced 3β-HSD expression until about 23 weeks gestation,[2—4] but premature delivery may result in a limited ability to produce cortisol.[26] In the current study, cortisol did not increase in preterm infants before late-onset AOP. However, before AOP onset, these infants had significantly higher levels of precursor hormones that are substrates for 3β-HSD, and they responded to hydrocortisone with resolution of clinical signs of adrenal insufficiency. These results indicate that the cause of late-onset AOP is undeveloped activity of 3β-HSD to produce cortisol under chronic stress after birth, and that the pathophysiology of late-onset AOP is likely a result of relative adrenal insufficiency.

It has been suggested that 11β-hydroxylase, which converts 11-deoxycortisol to cortisol, is the rate-limiting step of cortisol biosynthesis in preterm infants,[11,27] and that 11β-HSD types 1 and 2, which interconvert cortisone and cortisol, may also affect levels of circulating cortisol.[17] 17-OH progesterone is converted to 11-deoxycortiol, and 11-deoxycortisol, in turn, to cortisol. Reduced activity of 11β-hydroxylase in preterm infants has been described using the 11-deoxycortisol/cortisol ratio,[11] and low 11β-hydroxylase activity might explain the increased amount of 17-OH-progesterone in urinary steroid metabolites in preterm infants.[27] In the current study, the 17-OH-progesterone level and cortisol/17-OH-progesterone ratio did not differ between the two groups before AOP onset, showing that 11β-hydroxylase activity is not reduced in preterm infants with late-onset AOP. The activity of 11β-HSD types 1 and 2 in preterm infants might be changed with increasing

gestational age.[17] 11β-HSD is important for maintaining appropriate cortisol concentrations in cell and tissues, and high activity of 11β-HSD type 2, which converts cortisol to inactive cortisone, may persist in the fetal zone of the adrenal cortex in preterm infants.[28] However, other studies suggest that high activity of 11β-HSD type 2 does not persist in preterm infants of less than 32 weeks gestational age,[29] in agreement with our findings. In the current study, the cortisol/cortisone ratio also did not differ between the two groups before AOP onset, which suggests that 11β-HSD activity was not reduced in preterm infants with late-onset AOP.

In the current study, after the first week of life, concentrations of fetal zone steroids, consisting mainly of DHEA-S, continued to decrease as postmenstrual age increased. Cortisol concentrations also decreased gradually with increasing postmenstrual age, but without significance. All late-onset AOP occurred at less than 32 weeks postmenstrual age, and differences in cortisol biosynthesis between the two groups before onset of AOP subsequently resolved. These findings suggest that maturity of the adrenal cortex is related to postmenstrual age in preterm infants. Longitudinal studies have shown that urinary excretion of fetal zone steroids continues to decrease until full term and that fetal adrenal cortex involution is related to gestational age, rather than to birth,[18] in agreement with our results. In contrast, serum cortisol and DHEA-S rapidly decrease in the first week of life, regardless of gestational age, which suggests that fetal adrenal cortex involution is not related to gestational age.[19] Our results support findings suggesting a relationship between maturity of the adrenal cortex and gestational age. Most AOP cases occur within the first week of life and are transient, with adrenal function tending to return to normal by the end of the second week of life.[10] While this conclusion might be true for the population in this study, the median gestational age for infants with AOP was 28 weeks. As the population in our study was less mature than that in the previous study, adaptations of the adrenal cortex to extra-uterine life might occur over a longer period.

The fetal adrenal cortex contains little 3β-HSD and produces large amounts of DHEA-S.[1–4] In the current study, DHEA-S accounted for most of the total precursor hormones, and preterm infants with AOP had significantly higher DHEA-S (2- to 3-fold) before AOP onset, compared to preterm infants without AOP. The inverse association of biosynthesis of DHEA-S and 3β-HSD[1] suggests that onset of AOP might be affected by the fetal adrenal cortex. However, biosynthesis of cortisol is also important in preterm infants by converting pregnenolone into progesterone and 17-OH-progesterone via 3β-HSD and 17α-hydroxylase. Moreover, 11β-hydroxylase and 11β-HSD play important roles in subsequent rate-limiting steps,[11,17,27,28] and the HPA axis puts in motion these complex signaling pathways regulated by feedback and feedforward mechanisms.[4] Notably, circulating levels of cortisol and DHEA-S are important in response to acute and chronic stress, impact synaptic plasticity and neurogenesis, and may have agonist and antagonistic actions on several neurotransmitter systems.[4] In addition, the HPA axis can be dysregulated under chronic stress, resulting in changes in hormone levels and sensitivity.[30] Therefore, onset of AOP might be triggered by prolonged stress after birth, in addition to immaturity of the adrenal cortex.

The strengths of the study include the multiple and simultaneous GC/MS analysis of serum steroid hormones, and the linear mixed models based on longitudinal data. The concentrations of steroid hormones in preterm infants have been measured in several studies,[7,8,11,25] but rigorous evaluation has been difficult due to cross-reactivity in immunoassays. In this study, we used HPLC and GC/MS to avoid cross-reactivity. Steroid hormones involved in cortisol biosynthesis were also measured longitudinally, thus ensuring the reliability of the test for adrenocortical function, and linear mixed models were used for statistical analysis because sequential and multiple measurements for each infant should be clustered by subject and not handled as independent data.

The study has two limitations. First, the small sample size may lack the power to detect differences in some triggers of late-onset AOP. As early preterm births were accompanied by exacerbated symptoms, background factors such as gestational age and birth weight were slightly imbalanced due to the small target population. Therefore, we restricted initial samples to infants born before 29 weeks gestation to match the baseline characteristics of infants with and without AOP. A second limitation is that 11-deoxycortisol was not measured; therefore, we could not calculate the cortisol/11-deoxycortisol ratio to estimate 11β-hydroxylase activity. However, it is unlikely that this activity caused late-onset AOP because the concentrations of 17-OH-progesterone and cortisol/17-OH-progesterone ratios showed no differences between infants with and without late-onset AOP.

In conclusion, our results indicate that maturity of the adrenal cortex is related to postmenstrual age, and its function is immature in preterm infants who develop late-onset AOP until approximately 32 weeks postmenstrual age, resulting in relative adrenal insufficiency under stress. However, triggers for late-onset AOP are unclear and the small number of subjects in the current study makes this conclusion preliminary. Further studies of adrenal function in more infants are needed to facilitate earlier detection of AOP in the high-risk group, with the goal of improving the prognosis of preterm infants.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

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